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Persistent Organic Pollutants and the Association with Maternal and Infant Thyroid Homeostasis: A Multipollutant Assessment

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Abstract

Background: Disruption of thyroid homeostasis has been indicated in human studies targeting effects of persistent organic pollutants (POPs). Influence on the maternal thyroid system by POPs is of special interest during pregnancy, as such effects could impair infant thyroid homeostasis.

Objectives: We investigated the association between POPs and thyroid stimulating hormone (TSH) and thyroid hormones (THs) in mother and child pairs from the Northern Norway Mother-and-Child Contaminant Cohort Study (MISA).

Methods: Nineteen POPs and ten thyroid parameters were analysed in serum from 391 pregnant women in their second trimester. In addition, TSH concentrations in heel prick samples from the infants were analysed by the Norwegian Newborn Screening program. Association studies with a multipollutant approach were performed using multivariate analyses; partial least squares (PLS) regression, hierarchical clustering and principle component analysis (PCA).

Results: Several POPs were significantly associated to TSH and THs: i) PFOS was positively associated with TSH; ii) PCBs, HCB and nonachlors were inversely associated to T3, T4 and FT4; and, iii) PFDA and PFUnDA were inversely associated to T3 and FT3. After mutual adjustments for the other contaminants, only PFDA and PFUnDA remained significantly associated to T3 and FT3, respectively. Infants born by mothers within the highest TSH quartile had 10% higher mean concentrations of TSH compared to children born by mothers in the lowest TSH quartile.

Conclusion: The present results suggest that background exposures to POPs can alter maternal thyroid homeostasis. This research contributes to the understanding of multipollutant exposures using multivariate statistical approaches and highlights the complexity of investigating environmental concentrations and mixtures in regards to maternal and infant thyroid function.

Introduction

Human endocrine systems like the thyroid are susceptible to disruption by naturally-occurring and man-made compounds, possibly by affecting the hormone homeostasis. Endocrine disrupting abilities have been suggested for persistent organic pollutants (POPs), of which two major groups are perfluoroalkyl substances (PFASs) and organochlorines (OCs). POPs are persistent substances that have been emitted to the environment (Lohmann et al. 2007; Prevedouros et al. 2006). Still, PFASs and OCs have different chemical properties and histories of production and use. Diet is suspected to be the major current exposure pathway to POPs for humans (Malisch and Kotz. 2014; Vestergren and Cousins. 2009). In addition, PFASs are passed to humans through air, house dust, drinking water and water based beverages (Eschauzier et al. 2013; Haug et al. 2011; Ullah et al. 2011).

Disruption of thyroid homeostasis following POP exposure has been observed in animal experiments and indicated in human studies (Boas et al. 2012). Influence on the maternal thyroid system by POPs is of special interest during pregnancy, as such effects could delay and impair foetal and neonatal development (Massart and Meucci. 2007). The thyroid endocrine system is critical for regulating energy homeostasis, metabolic pathways and the growth and differentiation of many tissues and organs. Thyroid stimulation hormone (TSH) regulates the production of the thyroid hormones (THs), triiodothyronine (T3) and thyroxine (T4). Maternal T4 is the sole source of TH to the developing foetal brain before the onset of the foetal thyroid function at approximately 20 weeks gestation (Morreale De et al. 2004). The foetus is still dependent on maternal THs throughout the gestational period and inadequate transfer of these may alter thyroid homeostasis in infants also the first weeks after birth (Blackburn. 2013).

The major metabolic processes (e.g. metabolism of fat, glucose, protein and micro-nutrients) increase during the pregnancy along with an expansion of blood volume, to meet the demand of uterus and foetal development. During the first two trimesters of pregnancy, there are marked changes in the maternal HTP thyroid axis to increase the availability of THs in blood. In short, these changes lead to a two- to three-fold increase in thyroid hormone binding proteins (TH-BPs), and a subsequent decrease in levels of free thyroxine (FT4) and free triiodothyronine (FT3) followed by an increased production of T3 and T4. Changes in individual TH levels throughout pregnancy varies by gestational age, number of foetuses and study population, but generally, the woman achieves a new steady state in HTP function at the end of 2^{nd} trimester which is maintained until delivery (Blackburn. 2013). Pregnancy-induced changes in thyroid physiology affect laboratory interpretation and presently there are no universally accepted reference ranges (Fitzpatrick and Russell. 2010).

The potential influence on thyroid homeostasis by POPs in background exposed populations are of interest, especially if POPs can mimic or inhibit the response of natural hormones even at low doses (Vandenberg et al. 2012). Linear effects of many hormones exist up to a dose that occupies about 10% of receptors, at higher doses, occupancy rate does not linearly increase as the dose of the hormone increases (Welshons et al. 2003). Similar, some POPs might be more potent endocrine disruptors at low concentrations (Ruzzin et al. 2010). Therefore, the present study aimed to perform a multipollutant assessment of the effect of background exposures of POPs on thyroid homeostasis in mother-child pairs in Northern Norway.

Materials and methods

Study participants and collection of blood samples

The selected subjects in the present study were 391 women and child pairs from the Northern Norway Mother-and-Child Contaminant Cohort Study (MISA) which consists of 515 enrolled pregnant women, recruited from May 2007 to June 2009. The 391 participants with complete datasets consisting of maternal serum concentrations of PFASs, OCs, thyroid parameters and infant TSH concentrations were initially included in the study. Women with self-reported thyroid related disease (N=16) and/or twin pregnancy (N=6) were excluded and 370 mother and child pairs were included in the statistical analyses. The mothers answered a detailed questionnaire about diet and lifestyle at enrolment, and donated a blood sample during their second trimester (median gestational week 18). The women were requested to fast or eat a light, non-fatty breakfast no later than 2 hours before the blood sampling. Blood samples of infants were collected three days after birth. Detailed information about the study group characteristics, ethical approvals, the food frequency questionnaire (FFQ) and the blood collection procedures have been reported elsewhere (Hansen et al. 2010; Veyhe et al. 2012).

Chemical analyses

PFAS analyses

Blood samples were analysed for a broad selection of PFASs. A total of 26 PFASs were initially screened for in a sub-group of 50 serum samples. Compounds detected above the limit of detection (LOD) in more than 20% of the samples were further quantified in all serum samples. Detailed information about the compounds, sample preparation, extraction method, analytical method, reagents and instrumentation has been reported elsewhere (Berg et al. 2014; Hanssen et al. 2013). Briefly, PFASs were determined in serum samples using sonication-facilitated liquid–liquid extraction, activated ENVI-carb clean-up (Powley et al. 2005) and analysed by ultrahigh pressure liquid chromatography triple–quadrupole mass-spectrometry (UHPLC-MS/MS).

OC analyses

The methods employed for the OC analyses have been described in detail in Hansen et al. (2010). Briefly, internal standards, formic acid and deionised water were added to 2 ml serum sample and left in the fridge over night before being extracted through an HLB solid phase (SPE) column using dichloromethane. Further clean-up involved elution of compounds from Florisil columns with n-hexane/dichloromethane. OCs were identified and quantified in the extracts with a gas chromatograph/mass spectrometer operated in electron impact mode. Assessment of isotopic mass ratios, blank samples and standard reference materials ensured the quality of the results. Finally, lipids were detemined enzymatically and the summed amount of lipids was calculated as described by Akins et al. (1989).

The quality of the PFAS and OC analyses was assured through repetitive analysis of blank samples and reference samples. Additionally, our laboratory participates in the international Artic Monitoring and Assessment ring test programme for POPs in human serum (Institut national de santé publique du Québec. 2014). Interlaboratory comparisons and reference samples indicate that the uncertainties of our analyses are within ±15–20% of the assigned values. Further details on quality control issues are published elsewhere (Berg et al. 2014; Hansen et al. 2010). The linear perfluorooctane sulfonate (PFOS) isomers were chromatographically separated from the branched isomers and quantified separately. Summed concentrations of isomers were used in the statistical analyses.

TH and TH-BP analyses

Determination of maternal THs, TH-BPs (TBG, transthyretin (TTR) and albumin), thyroxine binding capacity and anti-TPO concentrations serum samples were performed by laboratory staff at the University Hospital of Northern Norway. The analyses are routine analyses used in the clinic for diagnostic purposes except for T3, T4 and thyroxine binding capacity. Analytical methods, instrumentation, analytical variation, quality controls and method specific reference ranges are reported by Berg et al. (2015). The laboratory is certified according to ISO 151810 (Norwegian accreditation. 2014). The Norwegian National Unit for Newborn Screening at Oslo University Hospital tested the newborn blood for TSH concentrations. Blood spots were collected on a S&S or Whatman 903 filter paper and analysed with Autodelfia neonatal TSH kits (PerkinElmer)

Statistical analyses

Statistical analyses were performed using SPSS statistic software, ver. 22 (IBM SPSS Inc. Chicago, IL, USA) and R (ver. 3.1.1; R Core Team). A statistical significance threshold of $p < 0.05$ was used. Only POPs with detection frequencies above 80% were evaluated in statistical models and concentrations below LODs were replaced by LOD/√2. All POP concentrations, TSH and THs were log-normally distributed (Shapiro–Wilk tests) and therefore log10-transformed in the statistical analyses. Spearman's ρ values were calculated for correlations. Statistical analyses were performed including POPs as ng/mL concentrations and repeated using mmol/L concentrations. Initially, partial least square (PLS) regressions were used to evaluate the impact of all POPs and potential covariates simultaneously on maternal serum concentrations of TSH and THs. Separate PLS models were performed with log10-transformed and standardized (z-scores) variables. For data reduction purposes and to increase the model predictive ability, only variables with variable importance to projection (VIP) values > 0.4 were included in the final model. For highly correlated covariates sensitive to pregnancy related changes, principal components analysis (PCA) was performed. The score of each woman on the first principal component was included as a common pregnancy vector in multiple linear regression models to avoid collinearity issues while adjusting for these factors. To minimize the number of contaminants to be included in linear regression models, hierarchical clustering analysis of POPs based on correlations (method: complete-linkage) was performed and groupings according to clusters were subsequently performed by simple addition of POP concentrations. Finally, contaminants (individual, grouped or summed, as ng/ml or mmol/L, assessed as quartiles) and covariates were included in multiple linear regression models to report the strength of associations between POPs with TSH and THs. Separate models were built for five dependent variables; TSH, T3, T4, FT3 and FT4 where the number of subjects varied between models (N=360-370) according to complete information sets. Diagnostic plots of the residuals and potential influential points were evaluated. Possible confounders where controlled by stratification on variables correlated to both PFOS and THs (results not presented).

Results

Population characteristics

Demographic characteristics of the pregnant women are summarized in Table 1 and present the variables used as covariates in final statistical models. Characteristics for other variables evaluated as covariates (including iodine status) were decribed in Berg et al. (2015). Demographic characteristics of the newborn are presented in Table 2, and includes available clinical data. The included subjects are representative for pregnant women from the geographical region. The demographic characteristics of the MISA study population and comparison to the general Norwegian pregnant population are reported in detail by Veyhe et al. (2012).

Contaminant concentrations and their correlations

Seven PFASs were detected in more than 80% of blood samples and were included in the statistical analyses; PFOS (median of 8.03 ng/mL) was the dominating compound followed by perfluorooctanoate (PFOA, 1.53 ng/mL), perfluorononanoate (PFNA, 0.56 ng/mL), perfluorohexane sulfonate (PFHxS, 0.44 ng/mL), perfluoroundecanoate (PFUnDA, 0.26 ng/mL), perfluorodecanoate (PFDA, 0.23 ng/mL) and perfluoroheptane sulfonate (PFHpS, 0.10 ng/mL). PFAS concentrations and their predictors are described in detail elsewhere (Berg et al. 2014).

Eight polychlorinated biphenyls (PCBs) and four pesticides were detected in more than 80% of blood samples and included in the statistical analyses (Supplemental Material, Table S1). The highest median wet-weight concentration were found for *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE, 0.24 ng/g) followed by PCB 153 (0.16 ng/g), PCB 180 (0.11 ng/g), PCB 138 (0.09 ng/g), hexachlorobenzene (HCB, 0.06 ng/g), PCB 170 (0.04 ng/g), PCB187 (0.03 ng/g), PCB118 (0.03 ng/g), PCB 163 (0.02 ng/g), *trans*-nonachlor (0.02 ng/g), PCB 99 (0.01 ng/g) and *cis*-nonachlor (0.004 ng/g). OC concentrations in the entire study population and predictors are described in detail by Veyhe et al. (2015).

The OCs intragroup correlations were higher within the OCs (ranging r=0.54-0.95) compared to the PFASs (ranging r=0.19-0.75) (see Supplemental Material, Table S2). In the hierarchical clustering analysis, the PCBs were separated in two separate groups and *cis*- and *trans*-nonachlors into one group (see Supplemental Material, Figure S1). The correlations between the

OCs and the PFASs were low and ranged from r=0.13-0.50 where the longest chained PFASs were more correlated to the OCs compared to the shorter chained compounds.

Concentrations of maternal TSH, THs and TH-BPs and infant TSH

Maternal concentrations of TSH, THs and TH-BPs were within non-pregnant reference ranges (Table 1) and time of blood sampling during the day did not influence the variance in concentrations between participants. Twenty two women had thyroid peroxidase antibodies above 34 IU/L and were categorized as anti-TPO positive according to the manufacturer. The anti-TPO positive women were included in all analyses, tables and figures as results were unchanged if excluding them. Concentrations of infant TSH are presented in Table 2. The ranges of infant TSH levels were within what is considered a normal reference range (Kapelari et al. 2008). Four infants could be classified with subclinical hypothyroidism as characterized by TSH concentrations > 5 mlU/L (Kaplowitz. 2010).

Maternal TH concentrations and associations with POP concentrations

PLS regression indicated positive associations between maternal concentrations of TSH and most PFASs and OCs (Fig. 1). Further, parity and thyroxine binding capacity were important covariates for TSH concentrations. The PLS regression also demonstrated an inverse relationship between several OCs, PFDA and PFUnDA with the THs (Fig. 1). Important covariates for these THs were variables related to the course of pregnancy: lipids, albumin, TBG, TTR, thyroxine binding capacity and gestational week and these were highly correlated. In the PCA, 50% of the variation in these variables was explained by the first principal component (PC) alone. This PC demonstrated the same association to the THs in the PLS regression plot as the individual variables (results not presented), but to avoid multicollinearity including all the individual variables in multiple regression models, individual PC scores were included as a "common pregnancy related vector".

Individual and grouped POPs were included in multiple linear regression models based on the hierarchical cluster analysis (details about the grouped compounds are reported in the Supplemental Material, Section A1) and demonstrated that maternal TSH concentrations were positively associated with PFOS, PCB groups and the nonachlor group (see Supplemental Material, Table S3). Further, there were negative associations between; i) T3 and PCB groups, HCB, the nonachlor group and PFDA; ii) T4 and PCB groups, HCB and the nonachlor group; iii) FT4 and PCB groups; and iv) FT3 and PFUnDA. However, with the exception of associations between PDFA and PFUnDA with T3 and FT3 (Table 3), associations between the individual and grouped contaminants and THs or TSH were no longer significant when including the other contaminants as covariates interchangeably. Finally, including all the contaminants as summed OCs (sumOCs), summed PFASs (sumPFASs) and summed OCs and PFASs (sumPOPs) in regression models (Table 3) demonstrated a positive association between sumPOPs and TSH, whereas sumOCs were inversely associated to T3, T4 and FT4.

The results from PLS regression, hierarchical cluster analysis and linear regressions were consistent regardless of including POP concentrations as either ng/mL or mmol/L (results not presented). Further, the regression coefficients for associations between OCs and THs were the same regardless of including wet-weight or lipid-adjusted OC concentrations in the models. However, the associations between OCs and T3, T4 and FT4 were stronger for wet-weight compared to lipid-adjusted concentrations. Parity and age were correlated to several POPs, TSH and THs, and repeating the analyses when stratifying on parity and age groups, demonstrated the same associations between POPs and TSH and THs as the full models (results not presented). As residual variance related to pregnancy-related changes could affect blood lipids, the analyses were repeated in a subset of women in gestational week 20 only (N=94). In these analyses, including the OCs as wet-weight or lipid-adjusted concentrations resulted in the same regression coefficients (results not presented).

Associations between levels of maternal TH and infant TSH

The infant TSH concentrations three days after birth were positively associated with maternal TSH concentrations in second trimester and inversely associated with maternal FT4 concentrations three days postpartum (Supplemental Material, Table S4). The children classified with subclinical hypothyroidism had mothers within the highest TSH and PFOS quartile. No associations between maternal POP concentrations with infant TSH concentrations or maternal POP and TH concentrations with birth outcomes were observed.

Discussion

Main findings

To our knowledge, this is the first multipollutant study investigating thyroid disrupting effects of both OCs and PFASs, including infant TSH concentrations and as many as ten thyroid hormone parameters in pregnant women. Background exposures of POPs influenced maternal concentrations of TSH and THs, whereas infant TSH concentrations were associated to maternal concentrations of TSH and FT4. The study results contribute to a comprehensive understanding of the maternal thyroid hormone homeostasis in relation to current composite POP concentrations.

Associations between concentrations of POPs and maternal TSH and THs

Several individual PFASs and OCs were associated to maternal TSH and THs, but only PFDA and PFUnDA were significantly associated to THs after adjusting for other POPs. We could therefore not separate the importance of most compounds as it is likely that they shared variance in TSH and TH concentrations in the linear regression models. Accordingly, including individual or grouped variables of PFASs, OCs and POPs in linear regression models, demonstrated the same overall associations to TSH and THs which is demonstrated by comparing Table 3 and Table S3. However, the strength of associations between individual POPs, mainly PFOS and PCBs with TSH, were stronger compared to between sumPOPs with TSH, and this may be explained by higher random variation in the latter regression equations. Concentrations of the PFASs were tenfold higher than the OCs and were highly correlated to the sumPOP variable ($r=0.98$) (Table 3). This likely explains why the results of regressions including sumPOPs resembled those for sumPFASs.

Recent literature report relevant mechanisms of thyroid disruption by POPs in general to be; i) disturbance of the overall activity of the thyroid gland by interference with the TH receptors; ii) stimulation or inhibition of enzyme functions which mediates iodine uptake of the thyroid gland in the synthesis of T3 and T4; and iii) competitive displacement of THs on their binding proteins (Boas et al. 2012). As the PFASs and OCs are indicated to be differentially associated to concentrations of THs, this could indicate different modes of action with regards to the effect of PFASs and OCs on the maternal thyroid homeostasis, but any conclusion is not feasible solely based on statistical associations. It has been hypothesized that PFOS can alter the thyroid hormone levels by competitive binding to TH-BPs, and does not affect the regulatory functions of the thyroid hormone system itself as is demonstrated for the OCs (Chang et al. 2008; Lau et al.

2007). However, the associations between OCs and T3, T4 and FT4 may also reflect a compensatory feedback mechanism of elevated TSH levels due to PFASs exposure, or the opposite, where TSH levels are elevated as a respons to disrepancies in TH levels due to disruption by OCs.

The influence of maternal POP exposures on infant thyroid function

We did not observe associations between maternal POP concentrations and concentrations of infant TSH. However, interpretation of TSH concentrations three days after birth in regards to dysregulation of infant TH homeostasis may be too early to indicate thyroid impairment, and that divergences in TH levels due to maternal POP exposures could develop throughout childhood. Indeed, associations between PCB concentrations in breastmilk and TH levels in 1-year old children have been demonstrated (Nagayama et al. 1998) and prenatal exposure to PCB and dioxin was reported to be associated with subtle cognitive and motor developmental delays in children at school age (Vreugdenhil et al. 2002). Still, in a different study PCBs 99, 138, 153, 180, 183, 187, 194, and 199 were positively associated with neonatal TSH concentrations measured three days after birth (Chevrier et al. 2007), while concentrations of T3 in three week old infant were inversely associated to low-chlorinated PCBs (Darnerud et al. 2010). The discrepancies between these studies and the present may be explained by different sampling periods between the studies, where women in the two latter studies were sampled in the years 1996-2000 and had threefold higher POP concentration compared to the present study population.

Multipollutant assessments of POPs

Hierarchical clustering demonstrated distinct clusters dividing the PFASs and OCs into separate groups. This is in line with their physicochemical properties, but may also partly reflect the difference in their concentrations and temporal trends (Nost et al. 2013; Nost et al. 2014). Stronger correlations within the OCs could indicate more homogenous exposure to the different OCs (Lohmann et al. 2007) compared to the PFASs, whereas stronger correlations between the longest chained PFASs and the OCs may reflect similar recent exposure routes and persistence for these compounds. Dallaire and colleagues (2009) reported comparable correlations between PFOS and OCs (r = 0.36-0.55 versus 0.25-0.45 in the present study) and between the different OCs (r = 0.69-0.98 versus 0.54-0.95) even though concentrations were higher and the sampling performed in 2004 in that study.

Assessing the impairment of physiological processes by contaminants is complicated by the complex correlation of exposures, as one specific POP that is associated in a given study may partly or largely reflect the influence of other POPs rather than the impact of that POP itself. Hence, we cannot exclude that the observed associations between POPs and concentrations of TSH and THs are related to other contaminants (e.g. brominated flame retardants, bisphenols and phthalates) not included in the statistical analyses. When assessing individual contaminants, we demonstrated significant associations between PFOS and TSH. Accordingly, PFOS was positively associated to TSH in pregnant women in Norway sampled in 1999-2008 (Wang et al. 2013). Further, we demonstrated that PCBs, HCB and nonachlors were inversely associated to T3, T4 and FT4. These results are in accordance to Chevrier et al. (2008) who reported inverse associations between OCs (e.g. PCBs and HCB) and T4 and FT4 in pregnant women sampled in 1999-2000, as well as Takser et al. (2005) who demonstrated inverse associations between concentrations of T3 and PCBs 138, 153 and 180. However, in the present study, the only associations between single contaminants and THs that remained significant when including the other contaminants as covariates were the associations between PFDA and PFUnDA with T3 and FT3, respectively (Table 3). In a number of other studies, authors have also been unable to conclude on the individual effect of OCs on TH status when controlling for other OCs because of the high intercorrelations between compounds (Dallaire et al. 2009; Chevrier et al. 2008; Takser et al. 2005). The latter studies therefore also applied summed OCs in their statistical models. The summing of POP groups in the present study were based on intra- and inter-group correlations of the POPs, hence we did not consider similar molecular mechanisms in the summing strategy. Finally, summing the concentrations of POPs assumes equal potencies and no synergistic effects, which may mask effects of individual compounds.

Clinical relevance

The 95% confidence interval for the maternal and infant thyroid parameters varied within what is considered normal reference ranges for healthy non-pregnant and infant populations (Norwegian Medical Association. 2015), thus the clinical relevance of the observations is not obvious. Still, infants born by mothers within the highest TSH quartile had 10% higher mean concentrations of TSH compared to children born by mothers in the lowest TSH quartile. This indicates an influence of maternal thyroid function on the infant TSH levels and could be a transferred effect of POP influence on the maternal thyroid homeostasis. Any disruption of maternal thyroid homeostasis during pregnancy and impairment in infant TH levels is indicated to affect infant development according to Morreale and colleagues (Morreale De et al. 2000; Morreale De et al. 2004). Maternal hypothyroidism with high TSH and low FT4 levels increases the risk of premature birth, preeclampsia, low birth weight and impaired neuropsychological development in childhood (Burman. 2009; Davis et al. 1988), whilst a decrease in maternal FT4 due to mild iodine deficiency may affect cognitive function of the offspring (Abalovich et al. 2007). Although a pregnant woman's hypothyroidism is subclinical (mild and asymptomatic) it can still influence foetal neurodevelopment (Berbel et al. 2009).

Strenghts and limitations

Due to the complexity of the thyroid system, especially during pregnancy, assessment of potential thyroid impairment cannot be interpreted solely from the individual thyroid parameters. Therefore, we included all major components in the maternal thyroid homeostasis. To account for the adaptations in metabolic processes in pregnant women a thorough assessment of pregnancy-related covariates were performed and several influenced the variation in TSH and TH concentrations. As variables (e.g. thyroxine binding capacity, TBG, TTR, lipids, and gestational week) influencing TH concentrations were highly correlated, they were included into a common pregnancy vector, thereby enabling us to adjust for all of them in multiple regressions instead of selecting individual ones. If these latter variables were not adjusted for in the statistical models (individually or included in the vector) many more PFASs and OCs were significantly associated with THs. Still, many of these covariates are not regarded in the majority of studies on POPs influence on THs in pregnant women.

When evaluating thyroid function in pregnant women, measurement of FT4 is recommended as free hormone reflects the physiological effects on thyroid hormones better than total hormone concentrations due to the pregnancy-related increases in TH-BPs (Fitzpatrick and Russell. 2010). Still, these changes could also mask an actual decrease in levels of T3, T4, FT3 and FT4. The natural interference on THs by physiological changes during pregnancy could be reduced if all the pregnant women were sampled at the exact same gestational week. As this was not possible in the present study, we included the TH-BPs, thyroxine binding capacity (reflects elevated levels of all the TH-BPs), lipids and gestational week as a proxy for the pregnancy related alterations in blood in statistical models. This was supported by repeated analyses of subset of women in gestational week 18 and 20 where the associations from full models were confirmed.

The wet-weight concentrations of OCs were used in the regression models to be comparable to the PFAS wet-weight concentrations. Due to the mutually dependency between OCs and THs with lipids (results not shown), we chose to use the wet weight concentrations in the regression models for THs. Further, total lipids were adjusted for in models included in the pregnancy vector and an additional adjustment for lipids of the OCs was not performed as it would probably have led to over adjustments. This was confirmed in analyses performed on women in gestational week 20, where the effect of pregnancy related variables was assumed similar, and where using either wet-weight or lipid adjusted concentrations gave the same regression coefficients.

An important problem in multiple statistical comparisons, is that the probability of wrongly concluding that there is at least one statistically significant effect across a set of tests, increases with each additional test (Gelman A et al. 2012). To minimize multiple comparisons, regression models in the present study were built on the overall associations from one PLS-regression model and not from several simple linear regression models.

Conclusions

The present study suggests that background exposure of POPs can alter thyroid hormone homeostasis in pregnant women, subsequently affecting the infant thyroid system. Our results highlight the challenges of assessing effects on thyroid function, especially during pregnancy due to the complexity of contaminant mixtures and the thyroid system. However, regarding the critical role of maternal thyroid hormones in foetal development, associations between maternal thyroid homeostasis with individual, grouped or summed POPs, are of great public health importance.

Reference List

Abalovich,M, Amino,N, Barbour,LA, Cobin,RH, De Groot,LJ, Glinoer,D et al. 2007. Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society Clinical Practice Guideline. J. Clin. Endocrinol. Metab. 92:S1-47.

Akins,JR, Waldrep,K, Bernert,JT, Jr. 1989. The estimation of total serum lipids by a completely enzymatic 'summation' method. Clin. Chim. Acta 184:219-226.

Berbel,P, Mestre,JL, Santamaria,A, Palazon,I, Franco,A, Graells,M et al. 2009. Delayed neurobehavioral development in children born to pregnant women with mild hypothyroxinemia during the first month of gestation: the importance of early iodine supplementation. Thyroid 19:511-519.

Berg,V, Nost,TH, Hansen,S, Elverland,A, Veyhe,AS, Jorde,R et al. 2015. Assessing the relationship between perfluoroalkyl substances, thyroid hormones and binding proteins in pregnant women; a longitudinal mixed effects approach. Environ. Int. 77:63-69.

Berg,V, Nost,TH, Huber,S, Rylander,C, Hansen,S, Veyhe,AS et al. 2014. Maternal serum concentrations of per- and polyfluoroalkyl substances and their predictors in years with reduced production and use. Environ. Int. 69:58-66.

Blackburn, S. T.Pituitary, Adrenal, and Thyroid function. In: Maternal, Fetal and Neonatal PhysiologyElsevier,2013;627-652.

Boas,M, Feldt-Rasmussen,U, Main,KM. 2012. Thyroid effects of endocrine disrupting chemicals. Mol. Cell Endocrinol. 355:240-248.

Burman,KD. 2009. Controversies surrounding pregnancy, maternal thyroid status, and fetal outcome. Thyroid 19:323-326.

Chang,SC, Thibodeaux,JR, Eastvold,ML, Ehresman,DJ, Bjork,JA, Froehlich,JW et al. 2008. Thyroid hormone status and pituitary function in adult rats given oral doses of perfluorooctanesulfonate (PFOS). Toxicology. 243:330-339.

Chevrier,J, Eskenazi,B, Bradman,A, Fenster,L, Barr,DB. 2007. Associations between prenatal exposure to polychlorinated biphenyls and neonatal thyroid-stimulating hormone levels in a Mexican-American population, Salinas Valley, California. Environ. Health. Perspect. 115:1490-1496.

Chevrier,J, Eskenazi,B, Holland,N, Bradman,A, Barr,DB. 2008. Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. Am. J. Epidemiol. 168:298-310.

Dallaire,R, Dewailly,E, Pereg,D, Dery,S, Ayotte,P. 2009. Thyroid function and plasma concentrations of polyhalogenated compounds in Inuit adults. Environ. Health Perspect. 117:1380-1386.

Darnerud,PO, Lignell,S, Glynn,A, Aune,M, Tornkvist,A, Stridsberg,M. 2010. POP levels in breast milk and maternal serum and thyroid hormone levels in mother-child pairs from Uppsala, Sweden. Environ. Int. 36:180-187.

Davis,LE, Leveno,KJ, Cunningham,FG. 1988. Hypothyroidism complicating pregnancy. Obstet. Gynecol. 72:108-112.

Eschauzier,C, Hoppe,M, Schlummer,M, de,VP. 2013. Presence and sources of anthropogenic perfluoroalkyl acids in high-consumption tap-water based beverages. Chemosphere 90:36-41.

Fitzpatrick,DL and Russell,MA. 2010. Diagnosis and management of thyroid disease in pregnancy. Obstet. Gynecol. Clin. North Am. 37:173-193.

Gelman A, Hill J, Yjima M. 2012. Why we (Usually) dont have to worry about multiple comparisons. Journal of Research on Educational Effectiveness 5:189-211.

Hansen,S, Nieboer,E, Odland,JO, Wilsgaard,T, Veyhe,AS, Sandanger,TM. 2010. Levels of organochlorines and lipids across pregnancy, delivery and postpartum periods in women from Northern Norway. J. Environ. Monit. 12:2128-2137.

Hanssen,L, Dudarev,AA, Huber,S, Odland,JO, Nieboer,E, Sandanger,TM. 2013. Partition of perfluoroalkyl substances (PFASs) in whole blood and plasma, assessed in maternal and umbilical cord samples from inhabitants of arctic Russia and Uzbekistan. Sci. Total Environ. 447:430-437.

Haug,LS, Huber,S, Becher,G, Thomsen,C. 2011. Characterisation of human exposure pathways to perfluorinated compounds--comparing exposure estimates with biomarkers of exposure. Environ. Int. 37:687-693.

Institut national de santé publique du Québec.2014. AMAP Ring Test for Persistent Organic Pollutants in Human Serum. Available: http://www.inspq.qc.ca/ctqenglish/eqas/amap/description [Accessed on 06 august 2015].

Kapelari,K, Kirchlechner,C, Hogler,W, Schweitzer,K, Virgolini,I, Moncayo,R. 2008. Pediatric reference intervals for thyroid hormone levels from birth to adulthood: a retrospective study. BMC. Endocr. Disord. 8:15.

Kaplowitz,PB. 2010. Subclinical hypothyroidism in children: normal variation or sign of a failing thyroid gland? Int. J. Pediatr. Endocrinol. 2010:281453.

Labquality Finland.2014. External quality assessment for medical laboratories. Available: http://www.labquality.fi/eqa-eqas/eqa-eqas-program-scheme/external-quality-assessment/ [Accessed on 06 august 2015].

Lau,C, Anitole,K, Hodes,C, Lai,D, Pfahles-Hutchens,A, Seed,J. 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci. 99:366-394.

Lohmann,R, Breivik,K, Dachs,J, Muir,D. 2007. Global fate of POPs: current and future research directions. Environ. Pollut. 150:150-165.

Malisch,R and Kotz,A. 2014. Dioxins and PCBs in feed and food--review from European perspective. Sci. Total. Environ. 491-492:2-10.

Massart,F and Meucci,V. 2007. Environmental thyroid toxicants and child endocrine health. Pediatr. Endocrinol. Rev. 5:500-509.

Morreale De,EG, Obregon,MJ, Escobar del,RF. 2000. Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia? J Clin. Endocrinol. Metab 85:3975-3987.

Morreale De,EG, Obregon,MJ, Escobar del,RF. 2004. Role of thyroid hormone during early brain development. Eur. J Endocrinol. 151 Suppl 3:U25-U37.

Nagayama,J, Okamura,K, Iida,T, Hirakawa,H, Matsueda,T, Tsuji,H et al. 1998. Postnatal exposure to chlorinated dioxins and related chemicals on thyroid hormone status in Japanese breast-fed infants. Chemosphere. 37:1789-1793.

Norwegian accreditation.2014. Norwegian accreditation. Available: http://www.akkreditert.no/en/hva-er-akkreditering/ [Accessed on 06 august 2015].

Norwegian Medical Association.2015. [National usermanual in Medical Biochemistry] Nasjonal brukerhåndbok i Medisinsk Biokjemi [In Norwegian]. Available: http://brukerhandboken.no/index.php?var1=aapne&bok_id=kliniskkjemi [Accessed on 05. august 2015].

Nost,TH, Breivik,K, Fuskevag,OM, Nieboer,E, Odland,JO, Sandanger,TM. 2013. Persistent organic pollutants in norwegian men from 1979 to 2007: intraindividual changes, age-period-cohort effects, and model predictions. Environ. Health Perspect. 121:1292-1298.

Nost,TH, Vestergren,R, Berg,V, Nieboer,E, Odland,JO, Sandanger,TM. 2014. Repeated measurements of per- and polyfluoroalkyl substances (PFASs) from 1979 to 2007 in males from Northern Norway: assessing time trends, compound correlations and relations to age/birth cohort. Environ. Int. 67:43-53.

Powley,CR, George,SW, Ryan,TW, Buck,RC. 2005. Matrix effect-free analytical methods for determination of perfluorinated carboxylic acids in environmental matrixes. Anal. Chem. 77:6353-6358.

Prevedouros,K, Cousins,IT, Buck,RC, Korzeniowski,SH. 2006. Sources, fate and transport of perfluorocarboxylates. Environ. Sci. Technol. 40:32-44.

Ruzzin,J, Petersen,R, Meugnier,E, Madsen,L, Lock,EJ, Lillefosse,H et al. 2010. Persistent organic pollutant exposure leads to insulin resistance syndrome. Environ. Health. Perspect. 118:465-471.

Takser,L, Mergler,D, Baldwin,M, de,GS, Smargiassi,A, Lafond,J. 2005. Thyroid hormones in pregnancy in relation to environmental exposure to organochlorine compounds and mercury. Environ. Health. Perspect. 113:1039-1045.

Ullah,S, Alsberg,T, Berger,U. 2011. Simultaneous determination of perfluoroalkyl phosphonates, carboxylates, and sulfonates in drinking water. J Chromatogr. A 1218:6388-6395.

Vandenberg,LN, Colborn,T, Hayes,TB, Heindel,JJ, Jacobs,DR, Jr., Lee,DH et al. 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr. Rev. 33:378-455.

Vestergren,R and Cousins,IT. 2009. Tracking the pathways of human exposure to perfluorocarboxylates. Environ. Sci. Technol. 43:5565-5575.

Veyhe,AS, Hansen,S, Sandanger,TM, Nieboer,E, Odland,JO. 2012. The Northern Norway mother-and-child contaminant cohort study: implementation, population characteristics and summary of dietary findings. Int J Circumpolar Health 71:18644.

Veyhe,AS, Hofoss,D, Hansen,S, Thomassen,Y, Sandanger,TM, Odland,JO et al. 2015. The Northern Norway Mother-and-Child Contaminant Cohort (MISA) Study: PCA analyses of environmental contaminants in maternal sera and dietary intake in early pregnancy. Int. J. Hyg. Environ. Health. 218:254-264.

Vreugdenhil,HJ, Lanting,CI, Mulder,PG, Boersma,ER, Weisglas-Kuperus,N. 2002. Effects of prenatal PCB and dioxin background exposure on cognitive and motor abilities in Dutch children at school age. J. Pediatr. 140:48-56.

Wang,Y, Starling,AP, Haug,LS, Eggesbo,M, Becher,G, Thomsen,C et al. 2013. Association between perfluoroalkyl substances and thyroid stimulating hormone among pregnant women: a cross-sectional study. Environ. Health 12:76.

Welshons,WV, Thayer,KA, Judy,BM, Taylor,JA, Curran,EM, vom Saal,FS. 2003. Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. Environ. Health. Perspect. 111:994-1006.

Table 1: Maternal concentrations[a] of THs, TH-BPs, thyroxin binding capacity and maternal characteristics (n= 370)

Variable	Median (Range)	Mean ± SD	Study pop ref range[b]
TSH (mlU/L)	1.55 (0.06, 10.2)	1.76 ± 1.04	0.44, 4.48
T3 (nmol/L)	2.71 (1.47, 4.75)	2.75 ± 0.46	1.97, 3.73
T4 (nmol/L)	145 (92.00, 215)	146 ± 21.1	111, 190
FT3 (pmol/L)	4.59 (2.99, 7.08)	4.62 ± 0.53	3.66, 5.79
FT4 (pmol/L)	13.0 (9.00, 20.0)	13.4 ± 1.62	10.0, 17.0
Thyroxin binding capacity [c]	1.26 (0.84, 1.50)	1.26 ± 0.09	1.07, 1.43
TBG (mg/L)	36.7 (23.2, 69.6)	37.2 ± 6.74	26.2, 53.3
TTR (g/L)	0.19 (0.09, 0.27)	0.19 ± 0.03	0.15, 0.25
Albumin (g/L)	40.0 (33.9, 47.4)	40.2 ± 2.42	36.0, 46.0
Total lipid (mg/dL)	672 (344, 1072)	672 ± 126	442, 943
Age	31 (18, 43)	31 ±5.0	
Parity	1 (0, 4)	1±1.0	
Prepregnancy BMI	23 (17, 40)	24.0 ±4.3	
BMI second trimester	25 (18, 43)	26.0 ±4.4	
Gestational week at blood sampling	18 (10, 34)	18.0 ±3.4	
Physical activity (prepregnancy)[d]	6.3 (1, 10)	6.0 ±1.7	
Pregnancy vector[e]	-0.01 (-2.5, 3.2)	-0.03 ±0.9	

[a]Anti-TPO positive women (n=22) are included in medians.

[b]Study population reference range defined as the 2.5 percentile (lower range) and 97.5 percentile (upper range) for this study population

[c]Unit in TBI = thyroxin binding index

[d]Reported degree of physical activity on a 1-10 point scale between very seldom to very often

[e]Common vector for pregnancy related variables, the vector scores includes thyroxin binding capacity, TBG, TTR, albumin, total lipids and gestational week. Se text for details.

Table 2. Infant TSH concentrations, infant characteristics and study population specific reference range (n= 370)

	Boys/girls	Median (Range)	AM[a] (SD)	Study pop ref range[b]
Gender	196/174			
TSH (mlU/L)		1.20 (0.07, 7.50)	1.32 (0.94)	0.13, 3.90
Gestational length (days)		282 (212, 299)	280 (10.5)	
Age at sampling (hours)		72 (48, 364)	74 (23.7)	
Birthweight (grams)		3595 (1330, 4930)	3626 (505)	
Head circumference (cm)		36 (27, 40)	35.6 (1.46)	
Length (cm)		50 (41, 57)	50.3 (2.10)	

[a]Arithmetic mean
[b]Study population reference range defined as the 2.5 percentile (lower range) and 97.5 percentile (upper range) for this infant population

Table 3. Associations[a] between serum concentrations of POPs with TSH and TH concentrations in pregnant women

Predictors	N	TSH mlU/L[b]	T3 nmol/L[c]	T4 nmol/L[d]	FT4 pmol/L[e]	FT3[e]
Model 1: SumOCs[f]						
Quartile 1: 0.18-0.63	90	-	Reference	Reference	Reference	-
Quartile 2: 0.64-0.83	90	-	-0.01 (-0.03, 0.01)	0.001 (-0.02, 0.02)	0.001 (-0.01, 0.02)	-
Quartile 3: 0.84-1.12	91	-	-0.01 (-0.03, 0.01)	-0.004 (-0.02, 0.02)	-0.01 (-0.02, 0.01)	-
Quartile 4: 1.13-4.65	90	-	-0.03 (-0.06, -0,01)*	-0.02 (-0.04, -0.003)*	-0.02 (-0.03, -0.001)*	-
Model 2: SumPFASs[g]						
Quartile 1: 1.01-8.32	90	-	-	-	-	-
Quartile 2: 8.33-11.7	90	-	-	-	-	-
Quartile 3: 11.8-15.2	91	-	-	-	-	-
Quartile 4: 15.3-45.4	90	-	-	-	-	-
Model 3: SumPOPs[h]						
Quartile 1: 1.77-9.00	90	Reference	-	-	-	-
Quartile 2: 9.01-12.5	90	0.04 (-0.03, 0.11)	-	-	-	-
Quartile 3: 12.6-16.5	91	0.06 (-0.02, 0.14)	-	-	-	-
Quartile 4: 16.6-46.7	90	0.08 (0.001, 0.16)*	-	-	-	-
Model 4: PFDA						
Quartile 1: 0.05-0.17	90	-	Reference	-	-	-
Quartile 2: 0.18-2.3	90	-	-0.01 (-0.030. 0.007)	-	-	-
Quartile 3: 0.24-0.31	91	-	-0.01 (-0.032, 0.005)	-	-	-
Quartile 4: 0.32-2.34	90	-	-0.02 (-0.044, -0.005)*	-	-	-
Model 5: PFUnDA						
Quartile 1: 0.02-0.16	90	-	-	-	-	Reference
Quartile 2: 0.17-0.26	90	-	-	-	-	-0.01 (-0.024, 0.004)
Quartile 3: 0.27-0.38	91	-	-	-	-	-0.01 (-0.024, 0.004)
Quartile 4: 0.39-1.46	90	-	-	-	-	-0.02 (-0.033, -0.003)*

*p<0.05, calculated for the change in concentrations compared to the reference quartile.

[a]Regression coefficient β, i.e. change in concentrations (100% x β) across quartiles with the lowest quartile as reference group.

[b]The model is adjusted for parity, T-uptake in addition to sumPFASs and sumOCs in TSH models 1 and 2, respectively.

[c]The model is adjusted for pregnancy related change vector, parity, age, BMI, physical activity and sumPFASs.

[d] The model is adjusted for pregnancy related change vector, age, physical activity and sumPFASs.

[e]The model is adjusted for pregnancy related change vector, age, BMI and sumPOPs.

[f]Includes PCB99,118,138,153,163,170,180,187, *p,p´*-DDE, HCB, *cis*- and *trans*-Nonachlor.

[g]Includes PFHpS, PFHxS, PFOA, PFOS, PFNA, PFDA and PFUnDA.

[h]Includes SumOCs and SumPFASs.

Figure 1: PLS loading plot for TSH and TH concentrations. The plot describes the linear relationship between the independent variables (contaminants and covariates) and the dependent variables (TSH and THs) and how the variables load onto the principal components. POPs circled with a dashed orange line were positively associated with TSH concentrations (marked with an orange circle). POPs boxed with a blue solid line were negatively associated with concentrations of T3, FT3, T4 and FT4 (marked with blue squares).

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Figure 1.